In vitro growth of Sempur (*Dillenia philippinensis* Rolfe) shoots in response to different types of plants growth regulators supplemented on MS medium

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Abstract. Plant tissue culture of Sempur (*Dillenia philippinensis* Rolfe) has not been widely reported. The previous study showed that BAP added to Murashige & Skoog (MS) basal medium promoted the growth of *D. philippinensis* shoot culture. This research aimed to investigate the growth of *D. philippinensis* shoot tip cultures on MS medium containing three types of cytokinins namely BAP, Kinetin, and 2-iP compared with Gibberelic acid (GA_3). The results showed that the type and concentrations of cytokinins significantly affected all growth parameters observed except the number of roots which was only influenced by cytokinin concentrations alone. However, different levels of GA_3 did not significantly affect all growth parameters observed. After eight weeks of culture, MS medium containing 1 mg/l 2-iP gave the best response for plant height, (3.68± 0.26 cm). The highest number of leaves (20.33±3.14) and nodes (6.67± 0.21) were found in MS medium containing 0.5 mg/l BAP. While the highest number of roots (1.83±0.17) was found in MS medium without the addition of cytokinins. The range of average values of plant height at different levels of GA_3 was 3.08-3.74 cm; the number of leaves was 5.1-7.5; the number of nodes was 3.9-5.4 and the number of roots was 1.0-2.8.

1. Introduction

Dillenia philippinensis Rolfe, which is commonly called as Elephant apple is originally from the Philippines [1]. This plant is subjected for conservation due to its written in the red list of IUCN [2]. Genus of *Dillenia* has many purposes such as for ornamental plant, their secondary compounds as herbal medicine [3-5], or pesticides [6], and its wood for houses [7]. Propagation of this plant is limited due to this plant is categorized as neglected species. Plant tissue culture of *D. philippinensis*, which is common technique for *in vitro* plant production for many species, has not been widely reported. The growth response of *D. philippinensis* shoot tip culture is influenced by culture media composition [8]. Plant growth regulators is one of the critical components added in growth media which has important function for growth and development of explants. Influence of plant growth regulators in promoting cell division and cell regeneration are required to stimulate growth and shoot multiplications. Cytokinins and Gibberellins are two most commonly supplements used as plant growth regulators on plant tissue culture technology for a number of plant species, including the woody plants. Combination of BAP and NAA was applied on *D. philippinensis* [9], media MS containing BAP, NAA and 2,4-D was also added to *D. indica* [10,11]. Gibberellins has not been used on this genus. Combination of BAP and Gibberellins

increases shoot multiplication on woody plants of Polish wild roses [12]. The type and the concentration of plant growth regulators used depend on the species or genotype of the plant [8], types of explants cultured, and the objective of the experiment [13]. The consideration to use certain type of plant growth regulators is also critical to have low production cost of transplants in mass propagation purposes.

These following three types of cytokinins are frequently used for plant tissue culture media are BAP (benzylaminopurine) synonym with 6-BAP, Benzyladenine or N^6-Benzyladenine; Kinetin synonym with 6-Furfurylaminopurine or N^6-Furfuryladenine; 2-iP synonym with 6-(γ,γ-Dimethylallyl amino) purine or N^6-(2-Isopentenyl) adenine. Cytokinins are subdividing into two major classes as natural such as 2-iP and synthetic groups such as BAP, and Kinetin as results of their structural rearrangement. Cytokinins functions in plant development were acting synergy with other hormones, most notably auxin, to regulate cell division and differentiation [14]. Gibberellic acid is synonym with GA_3 or Gibberellin A_3. GA_3 are also commonly used in the plant tissue culture media to accelerate growth of explants [4]. GA_3 have the main function in plant height control [16]. Many reports indicate that BAP and Kinetin are common cytokinins used to accelerate growth of shoot cultures in many plant species such as *Stevia rebaudiana* [17,18], and in woody plant species such as citrus and agarwood. Cytokinin can be applied on its own or combined with auxins [19-23].

In most species of woody plants, propagation from seed is constrained by heterozygosity, which produce non-uniform seedlings [24]. Propagation by cuttings produces seedlings in limited numbers of transplants due to the low percentage of growth and longer period for transplants to form roots before they are ready to plant [25]. This disadvantage can be overcome by the application of tissue culture technique. Research on the development of *in vitro* plant culture is important to hold because it may overcome problems such as a low percentage of seed germination. Our previous research on *D. philippinensis* showed that the percentage of seed germination was 4.3%, after 12 weeks of incubation on MS medium without addition of plant growth regulators [26]. Shoot tip explants were used in this study because they showed to have better growth responses [27] compared with node explants, both used for cytokinin and GA_3 treatment. Murashige and Skoog (MS) basal medium was used in this research because this medium gave better growth responses than Woody Plant Medium (WPM). Addition of 0.5 mg/l BAP promoted shoot length and number of leaves [26,27].

Propagation of *D. philippinensis* shoot tip culture was done using MS medium [28] containing BAP [26,27]. However, improvement of the shoot growth is still necessary because addition of BAP did not give significant response for plant height parameters, as well as for other growth parameters (number of leaves and nodes) resulting the best growth on the medium containing BAP at 0.5 mg/l [26,27]. Therefore, this research was developed to investigate growth of *D. philippinensis* shoot tip grown on MS medium containing different types of cytokinins compared with addition of Gibberelic acid (GA_3) at different concentrations. This study aimed to descript the response of growth of *D. philippinensis* shoots tip cultured on MS medium supplemented with three types of cytokinins, namely BAP, Kinetin and 2-iP at 0, 0.5, 1 and 2 mg/l, compared with addition of GA_3 at 0, 1, 2 and 3 mg/l.

2. Materials and Methods

2.1. Materials

The plant materials used were shoot culture stock with routine subculture at every eight weeks derived from seed explants of *D. philippinensis*, collection of Laboratory of Plant Cell and Tissue Culture, Plant Biotechnology Research Group of Research Center for Biotechnology, LIPI at Cibinong, West Java, Indonesia. All shoots were grown on Murashige & Skoog (MS) basal medium containing 0.5 mg/l BAP. Shoots culture of 2-3 months old, having more than four leaves with at least 3 cm long, were used as source of explant for the experiments. MS medium added with sugar, as well as plant growth regulators i.e. BAP, Kinetin, 2-iP, and GA_3 and solidified with agar gelling agent was utilized in this study.

2.2. Methods

Shoot tip explants were cut at 1.5-2.0cm long, then they were cultured on media treatment. For the first experiment, we used three different types of plant growth regulators (cytokinin), namely BAP, Kinetin

and 2-iP at 0, 0.5, 1 and 2 mg/l. The experiment was carried out with two factors (type and concentration of cytokinin) of completely randomized design with six replicates for each cytokinin treatment. For the second experiment we also used shoot tip explants grown on MS medium containing GA_3 at 0, 1, 2 and 3 mg/l. The experiment was carried out with a single factor (concentration of GA_3) of completely randomized design, but with eight replicates for each medium containing different GA_3 concentrations. No rooting medium used in this experiment.

All cultures were maintained in a culture room at 25-27°C with continuous white TL lamp lighting (1000-1400 lux). Growth parameters namely shoot height, number of leaves, number of nodes and number of roots, were recorded every week until eight weeks of culture. Data of each treatment after eight weeks of culture was analyzed with ANOVA two factor for cytokinins treatment and single factor for GA_3 treatment, followed with post-hoc test (Duncan Multiple Range Test) at 5% confidence level using DSAASTAT V1.1 software. Performance of *D. philippinensis* shoots after eight weeks of culture were documented.

3. Results and Discussion

3.1. Shoot height

The height of *D. philippinensis* shoot tip explants in MS medium containing four different types of plant growth regulators from null to eight weeks in culture is shown in figure 1. Most shoots increased in growth from the beginning of culture until eight weeks in culture. Addition of BAP at 0.5, 1 and 2 mg/l had similar growth compared to the shoots grown in MS medium without addition of BAP until 4 weeks in culture. However, addition of 1 and 2 mg/l BAP after 4 weeks reduced the height growth. Addition of low BAP level at 0.5 mg/l and without addition of BAP increased the growth (figure 1A). Likewise, with addition with Kinetin, after week-5 growth of shoots on medium containing 0.5 and 1 mg/l Kinetin was higher compared to that grown in the medium containing 2 mg/l Kinetin. Shoots grown in MS medium without Kinetin was the highest after 6 to 8 weeks after culture (figure 1B). Addition of 2-iP increased shoot height until 8 weeks in culture, in contrast to shoots grown on the medium without 2-iP height of shoots was reduced after six weeks of culture (figure 1C). Addition of cytokinins generally suppress shoot height but have more effect for inducing adventitious shoot formation by promoting cells proliferation in the shoot apical meristems and localized auxin in the peripheral meristem for modyfing apical dominance, then differentiating organs [29].

The addition of 3 mg/l GA_3 gave similar effect with no addition of GA_3, in contrast with the addition of 1 and 2 mg/l GA_3 which inhibited shoot height (figure 1D). This results could be related to the type of explants used in this experiment. Piqueras and Debergh [30] explained that using shoot tip explants in all growth regulators treatment seemed to give good influence at shoot height parameters, because of the apical dominance. The apical dominance occurred as a response to the direction of explant growth to apical shoot, barely growing lateral shoots until the end of the observation, except at the addition of BAP at low concentration (figure 1A).

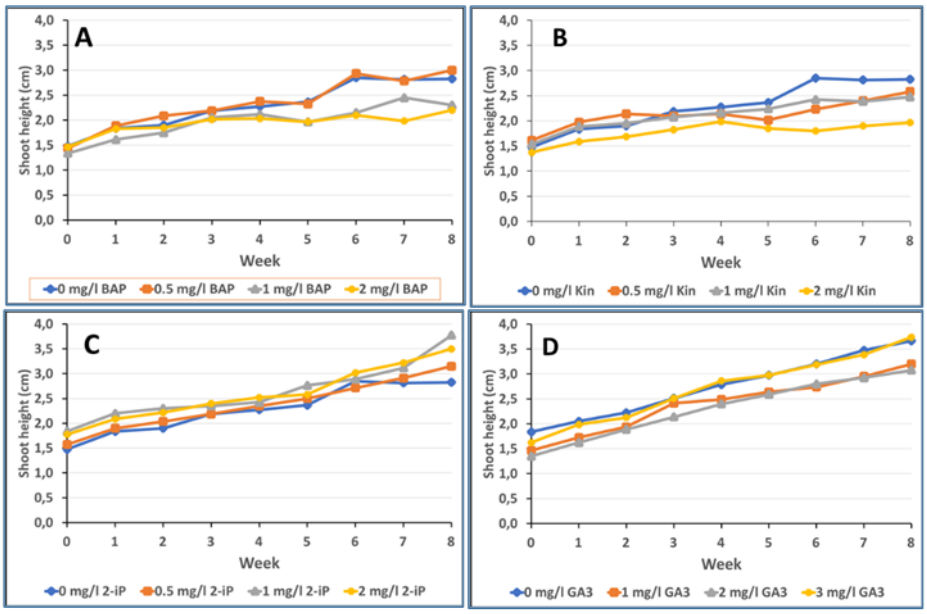

Figure 1. Shoot height of *Dillenia philippinensis in vitro* at 0-8 weeks cultured on MS medium containing BAP (A), Kinetin (B); 2-iP (C) at 0, 0.5, 1 and 2 mg/l and GA_3 (D) at 0, 1, 2 and 3 mg/l.

3.2. Leaf numbers

Addition of plant growth regulators affected leaf numbers of *D. philippinensis* shoot culture. Number of leaves from shoot tip explants cultured on MS medium containing four different kinds of plant growth regulators from week 0 to week 8 is shown in figure 2. After three weeks in culture, addition of different level of BAP gave different leaf numbers. Leaf numbers increased faster in the medium containing BAP at 0.5 and 1 mg/l. After 5 weeks in culture, the highest number of leaves was found in MS medium supplemented with 0.5 mg/l BAP, while no addition of BAP and addition of 2 mg/l BAP to the medium gave the lowest number of leaves (figure 2A). Low level of BAP (0.5 and 1 mg/l) gave best response for leaf numbers compared to Kinetin (Figure 2B), 2-iP (figure 2C), as well as GA_3 (figure 2D). Addition of 0.5 mg/l Kinetin gave slightly better growth of leaf numbers compared to higher concentrations of Kinetin (2 mg/l) after four weeks in culture. Medium without Kinetin did not enhance leaf numbers (figure 2B). Cytokinin 2-iP did not affect number of leaf (figure 2C). Gibberellic acid at 2 and 3 mg/l gave better response to leaf numbers from week-4 to week-8 of culture compared to lower concentration (1 mg/l) or without GA_3 (figure 2D).

The growth position of leaves on *D. philippinensis* node is alternate. One leaf is grown from each meristems node. Figure 2 shows that BAP treatment is able to induce the highest number of leaves. This result showed that BAP is a type of cytokinin that is quite responsive to organogenesis induction on *D. philippinensis in vitro* shoot culture. BAP and Kinetin are aromatic cytokinins type, according to its side chain of the chemical structure. 2-iP is isoprenoic cytokinins type. With this aromatic side chain, BAP has more effective biological activity but their efficiency depends on plant genotypes and other factors, so it requires several experiments to compare effectiveness as in the organogenesis in apple. For apple organogenesis, can be concluded that the most responsive cytokinins are TDZ and BA, while others (Zeatin, 2-iP, or Kinetin) are much less effective [31]. This result is similar to *D. philippinensis*.

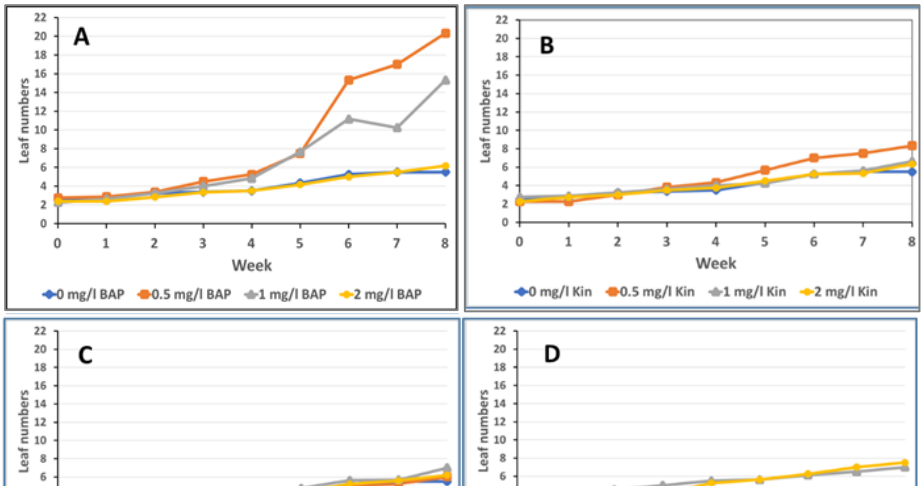

Figure 2. Leaf numbers of *Dillenia philippinensis in vitro* at 0-8 weeks cultured on MS medium containing BAP (A); Kinetin (B); 2-iP (C) at 0, 0.5, 1 and 2 mg/l and GA_3 (D) at 0, 1, 2 and 3 mg/l.

3.3. Node numbers

The node number of shoot tip explants cultured from week-0 to week-8, is shown in figure 3. The results showed that addition of BAP at 0.5 mg/l gave the best growth for node number compared to other BAP concentrations as well as other type of cytokinins (Kinetin, 2-iP) and GA_3 added to MS medium (figure 3). Addition of GA_3 did not enhance node numbers of *D. philippinensis* shoot tips because GA_3 will stimulate internode elongation [16]. Started from week-1 number of nodes only had slightly increase in all GA_3 concentrations tested (figure 3D). Nodes provide areas where the lateral bud is present from which the leaves, branches or flower buds start to grow. The more nodes formed the more leaf numbers will also be found. The mechanism of emergence of juvenile leaves from the nodes is regulated by signals intrinsic and extrinsic to the Shoot Apical Meristem (SAM). Tissue-specific manipulation of microRNA miR156 levels revealed that the SAM functions as an essential pool of miR156 early in shoot development, but that its effect on leaf identity declines with age [32].

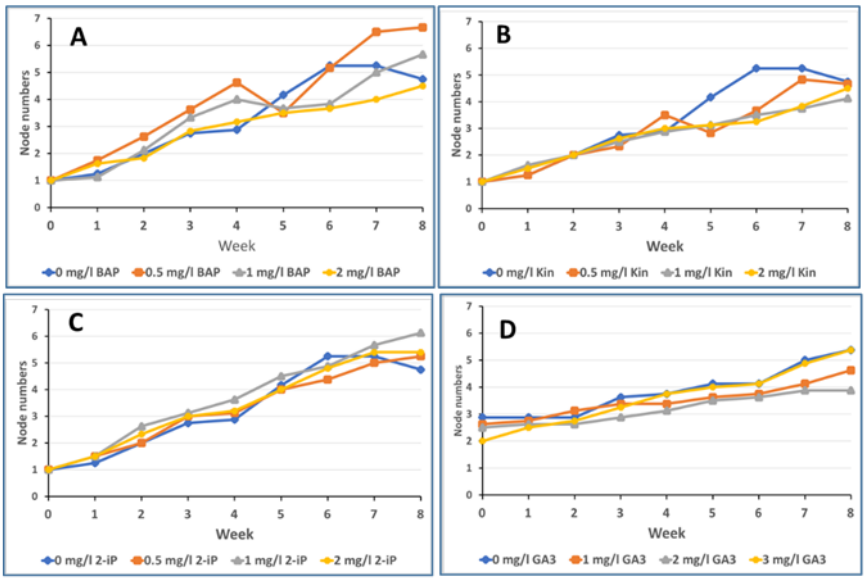

Figure 3. Node numbers of *Dillenia philippinensis in vitro* at 0-8 weeks cultured on MS medium containing BAP (A); Kinetin (B); 2-iP (C) at 0, 0.5, 1 and 2 mg/l and GA_3 (D) at 0, 1, 2 and 3 mg/l

3.4. Root numbers

Addition of cytokinins and GA_3 inhibited root formation of *D. philippinensis* as shown on figure 4. Only shoot tip explants grown on the medium without four types of plant growth regulators (control medium) increased root numbers after two weeks in culture. BAP completely inhibited root formation (figure 4A), Kinetin at low level (0.5 mg/l) slightly induce root growth after 4 weeks in culture (figure 4B), similarly with 2-iP (figure 4C). Research of apple rootstock stem cutting [33] and *in vitro* culture [34] gave a result that BAP addition in growth medium inhibited adventitious root development. Addition of GA_3 stimulated root growth but it affects lower than that of the control treatment (without the addition of GA_3) (figure 4D). This is in line with the explanation of the role of GA_3 in root growth. GA_3 is less required for rooting induction but rooting elongation depend on GA_3 [35]. Cytokinins play a role in the regulation of cell proliferation by influencing cell division and or cell differentiation. In shoots growth, cytokinins support cell proliferation including apical and axillary meristem activities but inhibit root growth. This is in accordance with the results of this study [14].

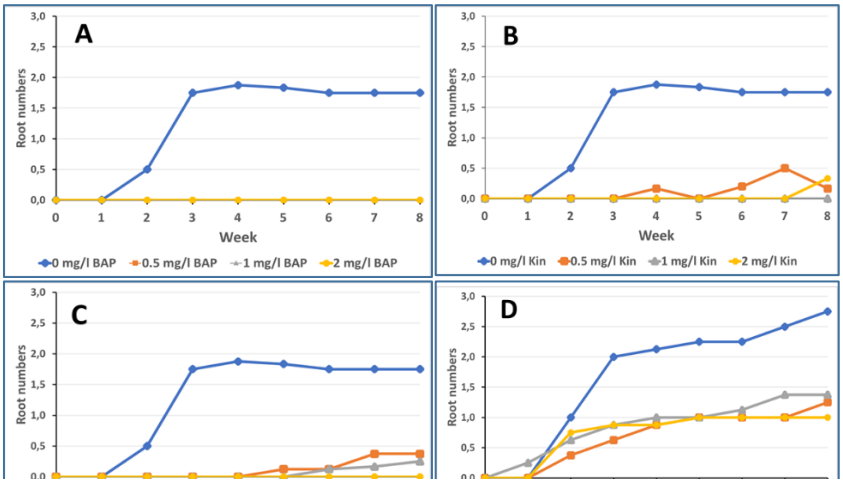

Figure 4. Root numbers of *Dillenia philippinensis in vitro* at 0-8 cultured on MS medium containing BAP (A); Kinetin (B); 2-iP (C) at 0, 0.5, 1 and 2 mg/l and GA_3 (D) at 0, 1, 2 and 3 mg/l.

3.5. Analysis of variance for all growth parameters tested after eight weeks of treatment

Results from the analysis of variance for the addition of three types of cytokinins, presented in table 1. Cytokinin types and concentration factors seemed to influence significantly on shoot height, leaf numbers and node numbers, but root numbers was only influenced significantly by cytokinins concentration factor. Therefore, three types of cytokinins added on MS medium did not significantly influence root numbers parameter. Concentrations gave significant influence on the shoot height. Table 2 shows the results from the analysis of variance for the addition of GA_3 treatment. Concentration of GA_3 added to MS medium gave significant influence on node and root numbers of explants, but not to shoots height and leaf numbers. This result showed that cytokinins (BAP, Kinetin, and 2-iP) had higher influence on shoot growth and development of *D. philippinensis* compared to GA_3 at concentrations tested. Roots development of *D. philippinensis* had no influence by cytokinin types, but affected by addition of GA_3.

Table 1. ANOVA of different type cytokinins treatment (BAP, Kinetin, and 2-iP) on their concentrations for shoot height, number of leaves, number of nodes and number of roots parameter of *Dillenia philippinensis in vitro* after eight weeks of treatment

No	Growth parameters	F value & Significanty			CV (%)
		Cytokinins Types	Concentrations	Cytokinins Types vs Concentrations	
1.	Shoot height	33,51**	3,08*	7,47**	13,62
2.	Number of leaves	18,19**	11,10**	6,72**	44,31
3.	Number of nodes	12,86**	4,78**	5,53**	13,76
4.	Number of roots	$0,75^{ns}$	66,82**	$0,61^{ns}$	81,09

Notes : * : significant on α: 5%; ** : highly significant; ns : not significant

Table 2. Anova of GA_3 at different concentrations on shoot height, number of leaves, number of nodes and number of roots of *Dillenia philippinensis* after eight weeks of treatment

No	Growth parameters	F value & Significanty Concentrations	CV (%)
1.	Shoot height	0.99 ^{ns}	27.36
2.	Number of leaves	2.14^{ns}	31.30
3.	Number of nodes	3.78*	21.68
4.	Number of roots	4.21*	67.98

Notes : * : significant at α = 5%; ** : highly significant; ns: not significant

3.6. Duncan Multiple Range test for significant parameters after eight weeks of treatment

Average value from growth response of *D. philippinensis* explant on significant influence parameters, was further analyzed with Duncan Multiple Range Test. Cytokinins and GA_3 treatment were analysed separately. The analysis results for cytokinins are shown in table 3, and for GA_3 is presented in table 4. Table 3 shows that the highest shoots found on MS medium containing 1 mg/l 2-iP, not significantly different with that of medium containing 2 mg/l 2-iP. The lowest shoots were found on the medium containing 2 mg/l Kinetin. The best medium for leaf numbers formation was MS containing 0.5 mg/l BAP. Higher level of BAP and Kinetin as well as 2-iP were not suitable to enhance formation of leaf. The leaf numbers corresponded with the number of nodes, therefore, the best medium for leaf formation was the same as for the node number development. All media without addition of cytokinins were best for root development as shown on table 3.

Table 3. Average of shoot height, number of leaves, number of nodes and number of roots *Dillenia philippinensis in vitro* culture after 8 weeks treatment with different types of cytokinins.

Cytokinin	Concen-tration (mg/l)	Shoot height (cm)	Number of leaves	Number of nodes	Number of roots
BAP	0	2,85 ± 0,06^{cde}	5,50 ± 0,22^{c}	4,83 ± 0,40^{cdef}	1,83 ± 0,17^{a}
	0,5	3,00 ± 0,20^{cd}	20,33 ± 3,14^{a}	6,67 ± 0,21^{a}	0,00 ± 0,00^{b}
	1	2,30 ± 0,13^{fg}	15,33 ± 3,37^{b}	5,67 ± 0,21^{bc}	0,00 ± 0,00^{b}
	2	2,20 ± 0,20^{fg}	6,17 ± 0,31^{c}	4,50 ± 0,34^{ef}	0,00 ± 0,00^{b}
Kinetin	0	2,85 ± 0,06^{cde}	5,50 ± 0,22^{c}	4,83 ± 0,40^{cdef}	1,83 ± 0,17^{a}
	0,5	2,58 ± 0,16^{def}	8,33 ± 2,03^{c}	4,67 ± 0,33^{def}	0,17 ± 0,17^{b}
	1	2,47 ± 0,09^{efg}	6,33 ± 0,61^{c}	4,17 ± 0,17^{f}	0,00 ± 0,00^{b}
	2	1,97 ± 0,21^{g}	6,33 ± 0,56^{c}	4,50 ± 0,22^{ef}	0,33 ± 0,33^{b}
2-iP	0	2,85 ± 0,06^{cde}	5,50 ± 0,22^{c}	4,83 ± 0,40^{cdef}	1,83 ± 0,17^{a}
	0,5	3,15 ± 0,16^{bc}	6,17 ± 0,17^{c}	5,33 ± 0,21^{bcde}	0,33 ± 0,33^{b}
	1	3,68 ± 0,26^{a}	6,67 ± 0,21^{c}	6,17 ± 0,17^{ab}	0,25 ± 0,25^{b}
	2	3,50 ± 0,10^{ab}	6,17 ± 0,17^{c}	5,50 ± 0,22^{bcd}	0,00 ± 0,00^{b}

Notes: Number followed with the same letters on the same column is not significantly different according to Duncan Multiple Range Test at α = 5%

Table 4 indicates that GA_3 concentrations did not affect the shoots height and number of leaves of *D. philippinensis* after eight weeks of culture. Addition of GA_3 reduced number of nodes and number of roots. The results show that GA_3 gave less influence for growth of *D. philippinensis*. However, height of *D. philippinensis* on MS medium containing GA_3 was similar to that grown on MS medium containing 2-iP (table 3 and figure 6). Leaf colour of shoots grown on the medium containing GA_3 was darker that that of shoots grown on other media added with cytokinins (figure 6). Darker leaf color caused by GA_3

treatment also occurred in various plants. One of them is the delay in yellowing of *Geranium* plant leaves. This showed that GA_3 are able to prevent leaf senescence, improve chlorophyll biosynthesis and reduce its catabolism. They preserve membrane integrity and antioxidant activity keeping ABA at low level [36].

Table 4. Average of shoots height, number of leaves, number of nodes and number of roots *Dillenia philippinensis in vitro* culture after 8 weeks treated with Gibberellic acid (GA_3).

GA_3 (mg/l)	Shoot height (cm)	Number of leaves	Number of nodes	Number of roots
0	3.66±0.17	5.1±0.29	5.4±0.18^{a}	2.8±0.41^{a}
1	3.20±0.23	6.1±0.74	4.6±0.32^{ab}	1.3±0.36^{b}
2	3.08±0.52	7.0±1.03	3.9±0.58^{b}	1.4±0.41^{b}
3	3.74±0.27	7.5±0.56	5.4±0.26^{a}	1.0±0.32^{b}

Notes: Number followed with the same letters on the same column is not significantly different according to Duncan Multiple Range Test at $\alpha = 5\%$.

Performance of *in vitro* shoot culture of *D. philippinensis* which growth on all media after eight weeks treatment is presented on figure 6, the initial shoot was shown on figure 5. At the beginning of culture, shoots started with two leaves, then developed to more leaves depending on the media compositions. After eight weeks, it shows that only BAP at 0.5 and 1 mg/l induced adventitious shoots. Leaves that formed adventitious shoots, had maximum number of 22 leaf blades after eight weeks of culture. At the early phases of aadventitious shoots growth, formation of new leaves can be seen clearly but internodes were short, which could not be easily recorded properly. However, on the medium containing GA_3, the increase number of leaves did not correspond to the increase of node numbers, as well as the increase of plant height. The addition of GA_3 indicated that vigorous shoot growth was found similar to that with 2-iP treatment (figure 6).

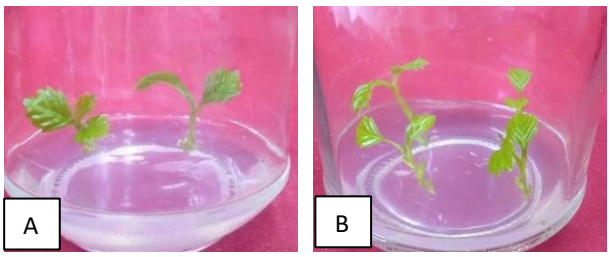

Figure 5. Performance of *in vitro* shoot culture of *Dillenia philippinensis* explants at the beginning of culture (0 week) for cytokinins treatment (A); for GA_3 treatment (B).

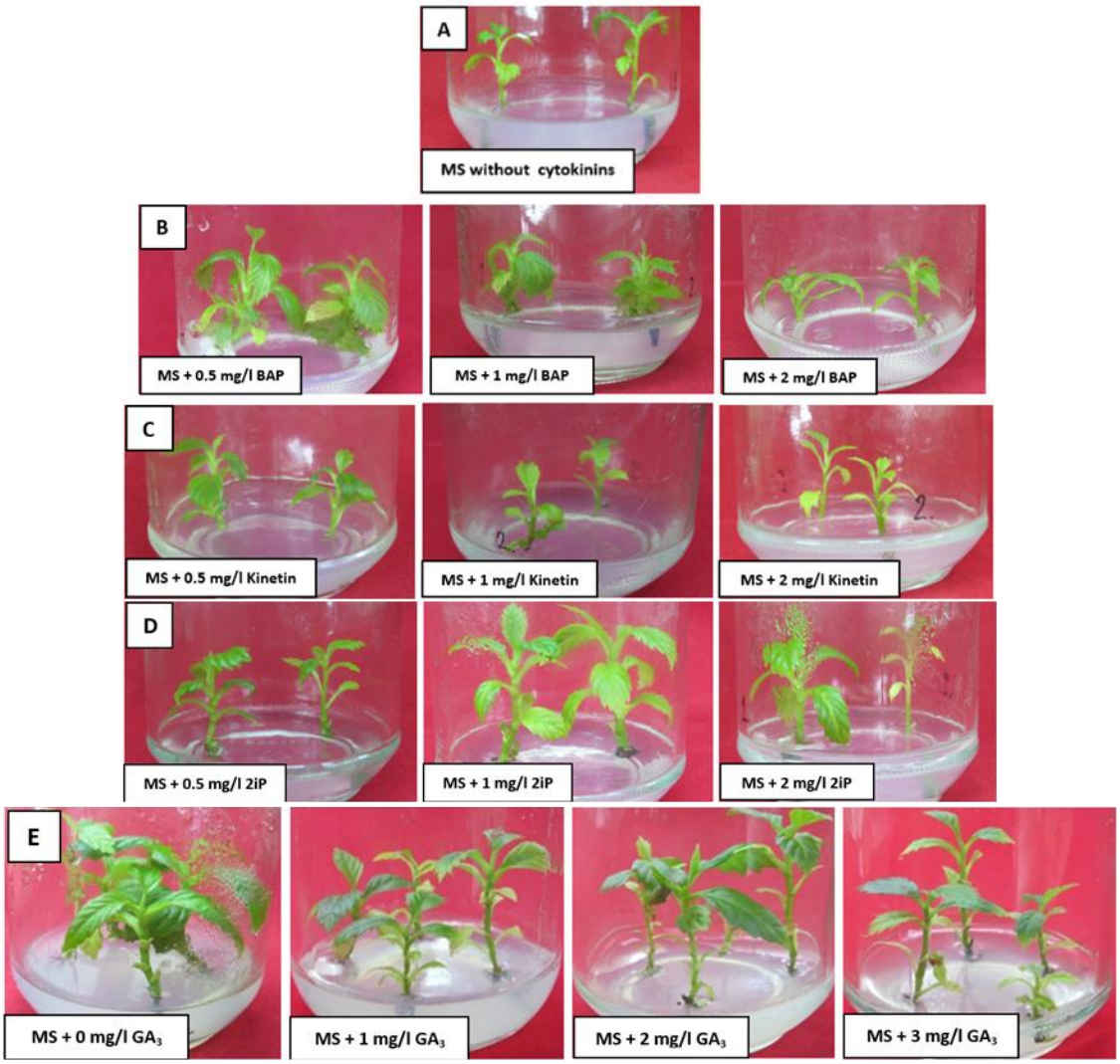

Figure 6. Performance of *in vitro* shoot culture of *Dillenia philippinensis* after eight weeks of cultured on control MS medium without plant growth regulators (A); MS medium supplemented with 0,5, 1 and 2 mg/l BAP (B), with 0,5, 1 and 2 mg/l Kinetin (C) and with 0,5, 1 and 2 mg/l 2-iP (D); MS medium with 0, 1, 2 and 3 mg/l GA_3 additions (E).

After eight weeks of culture, MS medium containing 1 mg/l 2-iP gives the best response for *D. philippinensis* plant height (3.68± 0.26 cm). The highest number of leaves (20.33±3.14) and nodes (6.67± 0.21) were found in MS medium containing 0.5 mg/l BAP. While the highest number of roots (1.83±0.17) was found in MS medium without the addition of cytokinin. The range of average values of plant height at a different level of GA_3 was 3.08-3.74 cm; the number of leaves was 5.1-7.5 leaves; the number of nodes was 3.9-5.4 nodes and the number of roots was 1.0-2.8 roots, respectively.

Addition of Kinetin and 2-iP in MS basic medium are compared with BAP. The level of concentration used is also less, difference with previous research by Wulandari *et al.* [27] to obtain a better understanding of the growth response after addition of cytokinins. It was proven that 2-iP resulted the best plant height response. In addition, 2-iP was showed to be suitable for inducing shoot multiplication and increasing rigidity at the same time. Addition with GA_3 most likely is not necessary, because it reaches plant heights at almost the same as the effect without addition GA_3, meaning that we provide low-cost production of transplants [16].

Addition of 2i-P at low concentrations, gave better response when compared with other cytokinins (Zeatin, BAP and Kinetin) on the parameters of survival, shoot formation, shoot length and number of leaves on *in vitro* establishment of 'Delite' Rabbiteye Blueberry microshoots [37]. The use of zeatin may be applied for *D. philippinensis* to have better understanding of its growth response on the medium with other type of cytokinin.

No lateral shoots were found until the end of the observation of our research. It is already known that some woody plants are less responsive to commonly used cytokinin such as Kinetin and BAP for lateral shoot formation. Addition of other supplements such as bio-regulatory substances polyamines, brassinosteroids, jasmonic acid and turgorins may be required. Beside that, other stronger cytokinins types may need to be tested [29].

The commercialized type of Gibberellic acid is GA_3 and has been widely applied to plant agriculture in general. Gibberellic acid has an important function in the internode elongation process. However, due to the complex biosynthetic pathway the exact bioactive sites of GA_3 synthesis in plants are unknown yet also levels concentration of GA_3 in plant cell are not well known, so understanding of the signal transduction pathway for stem and leaf elongation in different environmental factors is unknown yet [38]. This might be the reason that in our research of *D. philippinensis* had no significantly different response in explant growth parameters at GA_3 treatment.

4. Conclusions

In *D. philippinensis* shoot culture, each parameter of growth required different type and level of cytokinins. Elimination of cytokinin was best for root stimulation. MS medium containing 1 mg/l 2-iP gave the best response for plant height. The highest number of leaves were found in MS medium containing 0.5 mg/l BAP. This finding is useful for further research on enhancement of shoot multiplication to achieve higher production of plantlets.

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